ELSEVIER

Sleep Medicine 3 (2002) 377–378

SLEEP MEDICINE

www.elsevier.com/locate/sleep

Journal search and commentary

Article reviewed: Plasma orexin-A is lower in patients with narcolepsy ☆

Seiji Nishino*, Emmanuel Mignot

Stanford Sleep Research Center, Center for Narcolepsy, Stanford University School of Medicine, 701B Welch Road, 1st floor, Palo Alto, CA 94304, USA

Accepted 22 May 2002

Objectives

Determine plasma orexin-A (hypocretin 1) levels in patients with narcolepsy.

Study design

Clinical study.

Study population

Twelve Japanese narcoleptic patients met the criteria of narcolepsy of the International Classification of Sleep Disorders and age- and gender-matched controls. All 12 narcoleptic subjects are human leukocyte antigen (HLA) DR2 and DQB1*0602 positive, and 11 patients exhibit cataplexy. All controls were free of sleep, eating disorders and diabetes mellitus.

Methods

Blood samples were collected between 09:30 and 08:00 AM, after overnight fasting, and before breakfast. One ml of plasma was extracted using a reverse-phase SEP-PACK C 18 column and reconstituted in 0.25 ml of assay buffer. Orexin-A levels were measured with a commercially available radioimmunoassay (Peninsula Laboratories, San Carlos, CA).

Results

Plasma orexin-A in patients with narcolepsy ranged from 11 to 25 pg/ml, and the mean (±SD) plasma orexin-A levels of narcoleptic subjects, 20.83 (±4.34) pg/ml, were significantly lower ($t = 4.55$, d.f. $= 34$, $P < 0.0001$) than that of the control group (range 20–33 pg/ml; 26.67 ± 3.23 pg/ml). There was no relationship between plasma orexin-A concentrations and age, gender, body mass index, age at onset, or duration of illness. Orexin-A in 18 of the control subjects was measured twice, each time under identical conditions, 1–2 days apart, and the results of repeated sampling indicated that plasma orexin-A is a fairly stable trait within individuals.

Conclusions

Orexin-A (hypocretin-1) is detectable in the human plasma. Levels are significantly lower in narcolepsy versus age- and gender-matched normal controls. Low levels of orexin-A in plasma could serve as a biological marker for narcolepsy.

Comment

The recent discovery that human narcolepsy is associated with decreased hypocretin/orexin transmission in brain and cerebrospinal fluid (CSF) [1–3] is opening the way to novel diagnostic procedures. Most notably, decreased CSF hypocretin-1 (also called orexin-A) levels has been shown to be a highly specific and sensitive test for the diagnosis of narcolepsy, especially in cases with HLA-DQB1*0602 and cataplexy [4–7]. The finding has been confirmed independently by several other investigators [8,9] and is likely to be applicable in clinical practice. Although lumbar punctures are widely used in neurology, patients would clearly benefit if less invasive methods could be developed to evaluate hypocretrin/orexin deficiency. To address this issue, a flurry of recent publications has reported on the measurement of hypocretin/orexin-like immunoreactivity in human plasma under various conditions.

In this publication by Higuchi et al., plasma hypocretin-1/orexin-A levels were measured in patients with narcolepsy

☆ Higuchi S, Usui A, Murasaki M, Matsushita S, Nishioka N, Yoshino A, Matsui T, Muraoka H, Ishizuka Y, Kanba S, Sakurai T. Neurosci Lett 2002;318(2):61–64.

* Corresponding author. Tel.: +1-650-723-3724; fax: +1-650-725-4913.
E-mail address: nishino@leland.stanford.edu (S. Nishino).

PII: S1389-9457(02)00078-3

and found to be significantly lower than age- and gender-matched controls. However, the result is not consistent with the previous report by Dalal et al. [9] which did not detect any changes in plasma hypocretin-1/orexin-A levels in hypocretin/orexin deficient (CSF) narcolepsy. All published individual plasma levels, including these two studies, were never convincingly demonstrated to correspond to the genuine hypocretin/orexin signals. The immunoreactive (IR) plasma hypocretin-1/orexin-A levels in the narcoleptic and controls, reported by both Higuchi et al. and Dalal et al., are very close to the detection limit of the assay, and thus it is difficult to know whether the hypocretin/orexin signal can truly be distinguished from the assay background. Neither group has demonstrated that increasing the volume of blood applied results in a linear increase in the hypocretin/orexin signal detected (see evaluation of the CSF measuresments [10]). It is also not conclusive whether the signal reported reflects authentic hypocretin-1/orexin-A, peptide fragments or even other substances cross-reacting with the orexin-A antibody used (Peninsula Laboratories, San Carlos, CA). Arihara et al. [11] previously demonstrated that the major part of the IR hypocretin 1/orexin-A signal (using their own antibody) in the blood corresponds to the authentic hypocretin 1/orexin-A high-performance liquid chromatography (HPLC) fraction. However, the HPLC data was generated from a single pool of 30 ml of plasma samples (Takahashi, personal communication), and it was not certain whether IR levels (without HPLC purification), reported in each individual in various studies, reflected authentic hypocretin 1/orexin-A. In our laboratory, we have attempted to measure plasma hypocretin 1/orexin-A levels using the same extraction method, adding ethylenediaminetetra and aprotinin (a protease inhibitor) in the vacutaners and measuring the signal with another commercial antibody that we used for the CSF measures (Phoenix Pharmaceuticals, Belmont, CA). Larger initial volumes of plasma (10 ml) were used, but we could not detect a signal in the blood of ten controls and two narcoleptic subjects (<5 pg/ml). Thus, our results are also inconsistent with the studies by Higuchi et al. and Dalal et al., and rather point out the necessity for more detailed evaluations of the plasma assay and, similarly, an examination of whether the handling and method of the blood collection (i.e., by various anticoaglants and protein inhibitors) affect the hypocretin/orexin levels in the blood.

At the conceptual level, hypocretin/orexin are likely to exist in the blood. Finding decreased or normal levels in narcolepsy is, however, technically very difficult. Small amounts of peptides can leak from the brain to the periphery through the brain blood barrier. An increasing number of studies have demonstrated that hypocretin/orexin receptors are present in the periphery [12] and that low levels of hypocretins/orexin are likely to be produced peripherally [13]. All these factors could contribute to the existence of a small amount of hypocretin/orexin in plasma. If it is found that the major portion of hypocretin/orexin signals in the blood originates in the brain, and/or global (i.e., both central and peripheral) hypocretin/orexin deficiency exists in narcolepsy-cataplexy, then we may be able to detect low hypocretin 1/orexin-A levels in the blood. In this respect, the results presented in the current manuscript are very interesting. At the present time, however, the measures cannot be used diagnostically because the signal is likely to be partially masked by a high background. Although the mean plasma hypocretin 1/orexin-A level is significantly lower in narcolepsy, the sensibility to detect hypocretin/orexin deficiency in narcolepsy is low — only 25% (at 100% specificity).

Whether or not these results will be confirmed, we hope that our commentary will clarify the current state of affairs regarding hypocretin measurements in plasma. At this stage, it is still uncertain if the measured levels are genuine and if the test is sensitive enough to be used for any functional assays or diagnostically. However, the finding of Higuchi et al. is promising and deserves further study. If the major signal is real and the assay is improved in terms of the signal-noise ratio, plasma hypocretin 1/orexin-A measures might be used routinely at sleep clinics to diagnose narcolepsy.

References

[1] Nishino S, et al. Hypocretin (orexin) deficiency in human narcolepsy. Lancet 2000;355(9197):39–40.
[2] Peyron C, et al. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. Nat Med 2000;6(9):991–997.
[3] Thannickal TC, et al. Reduced number of hypocretin neurons in human narcolepsy. Neuron 2000;27(3):469–474.
[4] Nishino S, et al. Low CSF hypocretin (orexin) and altered energy homeostasis in human narcolepsy. Ann Neurol 2001;50:381–388.
[5] Ripley B, et al. CSF hypocretin/orexin levels in narcolepsy and other neurological conditions. Neurology 2001;57(12):2253–2258.
[6] Mignot E, et al. Clinical overlaps of the narcolepsy and hypocretin/orexin deficiency syndromes. Arch Neurol 2002 (in press).
[7] Kanbayashi T, et al. CSF hypocretin-1 (orexin-A) concentrations in narcolepsy with and without cataplexy and idiopathic hypersomnia. J Sleep Res 2002;11(1):91–93.
[8] Tsukamoto H, et al. Undetectable levels of CSF hypocretin-1 (orexin-A) in two prepubertal boys with narcolepsy. Neuropediatrics 2002;33(1):51–52.
[9] Dalal MA, et al. Normal plasma levels of orexin A (hypocretin-1) in narcoleptic patients. Neurology 2001;56(12):1749–1751 (see also Erratum in Neurology 2002;58(2):334).
[10] Ripley B, et al. Hypocretin levels in sporadic and familial cases of canine narcolepsy. Neurobiol Dis 2001;8(3):525–534.
[11] Arihara Z, et al. Immunoreactive orexin-A in human plasma. Peptides 2001;22(1):139–142.
[12] Johren O, et al. Prepro-orexin and orexin receptor mRNAs are differentially expressed in peripheral tissues of male and female rats. Endocrinology 2001;142(8):3324–3331.
[13] Kirchgessner AL, Liu M. Orexin synthesis and response in the gut. Neuron 1999;24(4):941–951.